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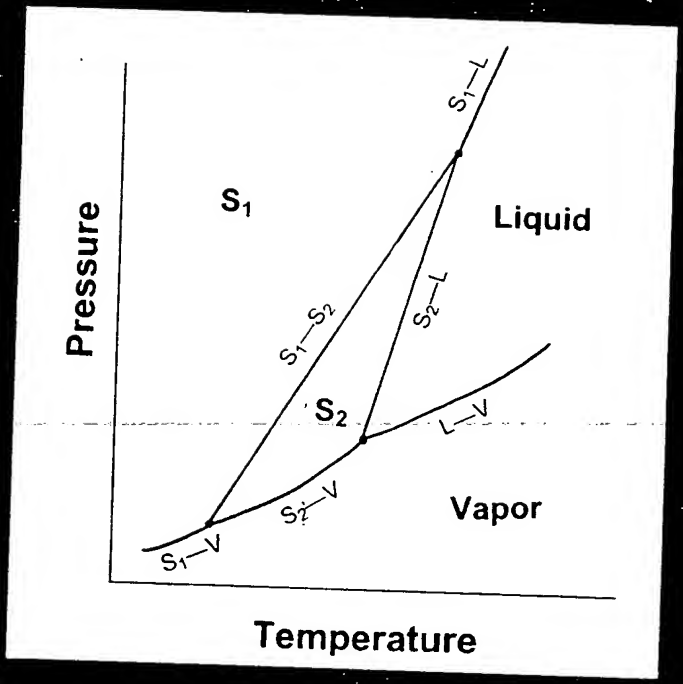
DRUGS AND THE PHARMACEUTICAL SCIENCES VOLUME 95

Polymorphism in Pharmaceutical Solids



edited by
Harry G. Brittain

Polymorphism in Pharmaceutical Solids

edited by
Harry G. Brittain
Discovery Laboratories, Inc.
Milford, New Jersey

MARCEL DEKKER, INC.

NEW YORK · BASEL

ISBN: 0-8247-0237-9

This book is printed on acid-free paper.

Headquarters
Marcel Dekker, Inc.
270 Madison Avenue, New York, NY 10016
tel: 212-696-9000; fax: 212-685-4540

Eastern Hemisphere Distribution
Marcel Dekker AG
Hutgasse 4, Postfach 812, CH-4001 Basel, Switzerland
tel: 44-61-261-8482; fax: 44-61-261-8896

World Wide Web
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Current printing (last digit):
10 9 8 7 6 5 4 3 2

PRINTED IN THE UNITED STATES OF AMERICA

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IV. METHODS EMPLOYED TO OBTAIN AMORPHOUS MATERIALS

Solids can exist in crystalline or amorphous form. Crystalline materials have defined structures, stoichiometric compositions, and melting points and are characterized by their chemical, thermal, electrical, optical, and mechanical properties [83]. By contrast, amorphous materials have no clearly defined molecular structure and no long-range order, so their structure can be viewed as being similar to that of a frozen liquid but without the thermal fluctuations observed in the liquid phase. As a result, amorphous materials exhibit the classical diffuse "halo" x-ray powder diffraction pattern rather than the sharp peaks observed in the pattern of a crystalline substance. When the halo is broad, it is often difficult to distinguish between a material that is truly amorphous (e.g., a true glass) and one that is merely microcrystalline. This situation exists because when microcrystallites have diameters less than about 50 Å in diameter, a similar "halo" effect is observed.

While crystalline solids offer the advantages of chemical and thermodynamic stability, amorphous solids are occasionally preferred because they undergo dissolution at a faster rate. Rapid dissolution is desirable in the case of solids, which must be dissolved prior to paren-

teral administration. Faster dissolution is also important for poorly soluble compounds administered orally, since there is often a correlation between dissolution rate and bioavailability. In fact, there are instances in which only the amorphous form has adequate bioavailability.

Amorphous solids can be precipitated from solution or obtained from melts of compounds by carrying out the solidification in such a way as to avoid the thermodynamically preferred crystallization process. They also can be prepared by disrupting an existing crystal structure. Excess free energy and entropy are incorporated into solids as they are converted into the amorphous state, since solidification occurs without permitting the molecules to reach their lowest energy states.

A. Solidification of the Melt

Amorphous solids are often created by rapidly cooling a liquid so that crystallization nuclei can neither be created nor grow sufficiently, whereupon the liquid then remains in the fluid state well below the normal freezing point. In principle, a liquid should freeze (crystallize) when cooled to a temperature below its freezing point. However, if the rate of cooling is high relative to the rate of crystallization, then the liquid state can persist well below the normal freezing point. As cooling continues there is a rise in the rate of increase of the viscosity of the supercooled liquid per unit drop in temperature. The initially mobile fluid turns into a syrup, then into a viscoelastic state, and finally into a brittle glass. A glass is, therefore, a supercooled liquid, and is characterized by an extremely high viscosity (typically of the order of 1014 Pa · s). Mechanically, if not structurally, glasses can be regarded as solids.

The characteristic temperature below which melted solids must be cooled to form a glass is the glass transition temperature T_g. The glass transition is a dynamic event that occurs at a temperature below which coordinated molecular motion becomes so slow that a liquid can be considered to take on the properties of a solid. While the exact value of this transition temperature depends on the heating rate, the glass transition temperature is generally found to be about two-thirds that of the melting temperature T_m. Glass transition temperatures reported for pharmaceuticals also follow this general rule, as can be seen in the

ATTACHMENT 2

5

Generation of Polymorphs, Hydrates, Solvates, and Amorphous Solids

J. Keith Guillory

The University of Iowa
Iowa City, Iowa

water occur. The monohydrate phase can be formed by exposing the anhydrous form to 98% relative humidity for ten days at 24°C [70].

III. METHODS EMPLOYED TO OBTAIN SOLVATE FORMS

Often, when solvents are employed in the purification of new drug substances by recrystallization, it is observed that the isolated crystals include solvent molecules, either entrapped within empty spaces in the lattice or interacting via hydrogen bonding or van der Waals force with molecules constituting the crystal lattice. Solvent molecules also can be found in close association with metal ions, completing the coordination sphere of the metal atom. Coordinated solvent molecules are considered as part of the crystallized molecule. A crystal with large empty channels or cavities is not stable because of packing demands. The size and chemical environment of the cavity or channel determine what kind of solvent molecule can be included in the structure and what kind of interaction occurs between solvent and structure.

Depending on the nature of molecular packing arrangements, it may happen that the inclusion of solvent is necessary to build a stable crystal structure. van Geerestein et al. [71] found during numerous crystallization attempts of 11β-[4-(dimethylamino)phenyl]-17β-hydroxy-17α-(I-propynyl) estra-4,9-diene-3-one] that crystals were only obtainable in the presence of *n*-butyl acetate or *n*-propyl acetate. The crystal structure of the compound crystallized from *n*-butyl acetate/methylcyclohexane was solved, and one solvent molecule was found in the crystal structure that showed no strong interactions with the rest of the structure. Apparently, this solvent molecule was necessary to fill empty space resulting after the molecular packing. Solvates in which the solvent fills empty space are generally nonstoichiometric, such as the nonstoichiometric solvates formed by droloxifene citrate with acetonitrile, 2-propanol, ethanol, 1-propanol, and 1-butanol. Typically such solvates exhibit the same x-ray diffraction pattern as does the nonsolvated compound.

When solvent molecules increase the strength of the crystal lattice, they can affect the stability of the compound to solid-state decom-

position. It has been observed that the four solvated and one nonsolvated structures of prenisolone *tert*-butyl acetate affect the flexibility of the steroid nucleus and the structure-dependent degradation of the compound when exposed to air and light [72].

van der Sluis and Kroon found 1,247 different compounds with cocrystallized solvents in the Cambridge Crystallographic Database [73]. Out of 46,460 total structures, they found 9,464 solvate structures, and 95% of these contained one of the 15 solvents given in Table 2.

The most commonly encountered solvates among pharmaceuticals are those of 1:1 stoichiometry, but occasionally mixed solvate species are encountered. For structures containing more than one solvent type, one generally finds nonpolar solvents crystallizing together on the one hand and polar solvents on the other. For example, the most common solvents found cocrystallizing with water are (in order of im-

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Ethanol	2.6
Tetrahydrofuran	2.3
Toluene	2.2
Acetonitrile	1.9
N,N-dimethylformamide	0.9
Diethyl ether	0.9
Pyridine	0.7
Dimethyl sulfoxide	0.5
Dioxane	0.5

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The techniques used to obtain solvates are generally similar to the solvent methods used to obtain polymorphs, i.e. crystallization from a single solvent, from mixed solvents, or by vapor diffusion. Sometimes, it is possible to exchange one solvent within the crystal structure for another. When one recrystallizes a hydrate from dry methanol, in most cases one is left with either a methanol solvate or an anhydrous, unsolvated form of the compound.

A large number of solvates have been reported, especially for steroids and antibiotics. It has been observed that cortisone acetate and dexamethasone acetate can be crystallized as 10 different solvates. Dirithromycin, a semisynthetic macrolide antibiotic, crystallizes in two anhydrous polymorphic forms and in at least nine stoichiometric solvate forms. Six of the known solvates are isomorphic, having nearly identical x-ray powder diffraction patterns [76]. In addition to the anhydrate and dihydrate, erythromycin also forms solvates with acetone, chloroform, ethanol, *n*-butanol, and *i*-propanol [77].

It may be instructive to consider some examples of solvate formation. The compound 5-methoxysulphadiazine forms 1:1 host–guest solvates with dioxane, chloroform, and tetrahydrofuran [78]. These were prepared by heating to boiling a solution of the sulfonamide in the appropriate solvent, followed by slow cooling to obtain large crystals. Spironolactone forms 1:1 solvates with methanol, ethanol, ethyl acetate, and benzene. It also forms a 2:1 spironolactone–acetonitrile solvate [79,80]. The spironolactone solvates were prepared by crystallization in a refrigerator from solutions that were nearly saturated at room temperature.

Another steroid that forms solvates is stanozolol [81]. Solvates having 1:1 stoichiometry were prepared by recrystallization from methanol, ethanol, and 2-propanol, by heating the compound in the

appropriate solvent to 60–70°C and then cooling to 0°C in an ice bath to induce crystallization. The compound also forms a monohydrate and two polymorphs. The polymorphs were prepared by heating the solvates to either 130°C (Form II) or 205°C (Form I).

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The arguments just provided detail the potential issues around hydrates in the development process. The other consideration is the frequency with which hydrates are encountered in real life. Focusing on active drug substances, it is estimated that approximately one-third of the pharmaceutical actives are capable of forming crystalline hydrates [3]. A search of the Cambridge Structural Database (CSD) shows that approximately 11% of all the reported crystal structures contain molecular water [4]. This represents over 16,000 compounds. If organometallic compounds are excluded, this number drops to approximately 6,000 (3.8%), and the breakdown of these according to hydration number is shown in Fig. 1. This shows the expected trend in which monohydrates are most frequently encountered, and where the frequency decreases almost exponentially as the hydration number increases. The hemihydrate stoichiometry occurs approximately as frequently as the trihydrate, which should serve as a caution to explore fully the occurrence of fractional hydration. That is, an apparent stoichiometry of 0.6 water molecules could be a partially dehydrated monohydrate, or it

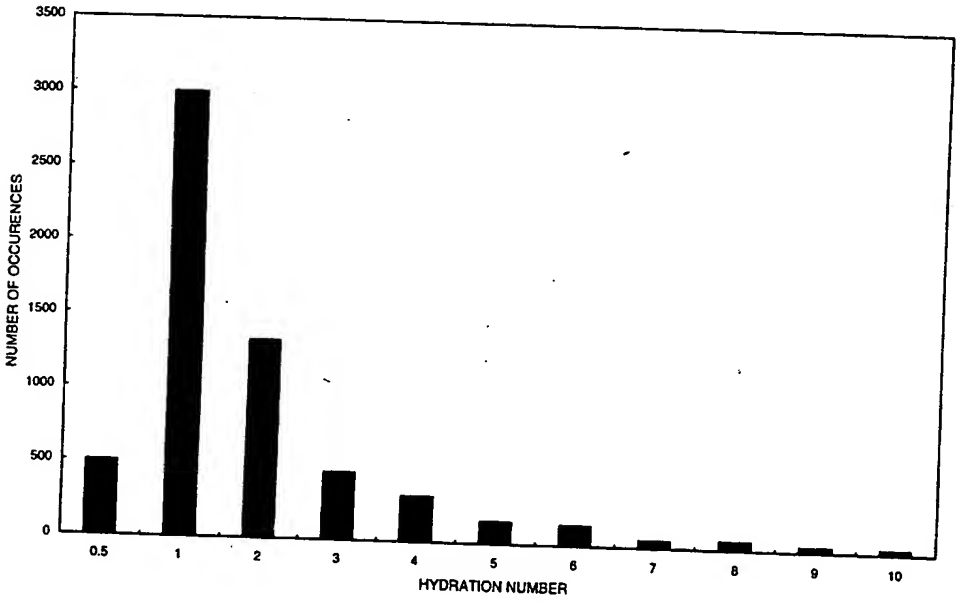

Fig. 1 Occurrence of various crystalline hydrate stoichiometries.

could be a hemihydrate with additional sorption due to defects or amorphous material.

The symmetry of these hydrate crystals follows fairly closely with that reported for organic structures overall [5]. Table 1 shows the breakdown for space groups, organized by crystal system, accounting for the top approximately 90% of the structures. $P2_1/c$ (number 14) is the most common space group here as with the general population of organic molecules contained in the CSD. It has been reported for inorganic species that hydrated structures are generally of lower symmetry than are their anhydrous counterparts [6]. This is attributed to the fact that the highest symmetry associated with the water molecule is C_{2v} and most inorganic structures are of higher symmetry. This is not obviously the case for organic structures. Regardless of the solvation state, organic molecules generally exhibit lower symmetry than do inorganic compounds, so the impact of the symmetry constraints imposed by water does not appear to be the controlling element. Further comparisons would be required to explore the phenomena fully.

Table 1 Space Groups for the Top 90% of Organic Crystalline Hydrates in the Cambridge Structural Database

Space group	Crystal system	Percent occurrence
P_{-1}	Triclinic	15.5
P_1	Triclinic	2.6
$P_{21/c}$	Monoclinic	23.2
P_{21}	Monoclinic	13.4
$C_{2/c1}$	Monoclinic	5.8
C_2	Monoclinic	2.8
P_{212121}	Orthorhombic	17.8
P_{bca}	Orthorhombic	2.3
P_{21212}	Orthorhombic	1.8
P_{na21}	Orthorhombic	1.8
P_{nma}	Orthorhombic	1.3
Unknown		1.2

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monohydrate conversion is 87°C [29]. As illustrated in Fig. 10, the pressure–temperature phase diagram of the system consists of three discrete dissociation curves that intersect at the ordinary transition points.

When dehydrating the trihydrate phase at constant temperature, the pressure would be maintained at the value corresponding to the dissociation pressure of the trihydrate until the complete disappearance of that phase. At that point, the pressure would fall to that characteristic pressure of the dihydrate phase. Continued dehydration would take place at the dissociation pressure of the dihydrate phase until it was completely transformed to the monohydrate, whereupon the pressure would immediately fall to the dissociation pressure of the monohydrate. As previously discussed, once the external pressure on a given hydrate is reduced below its characteristic dissociation pressure, the solid will undergo spontaneous efflorescence to a lower hydration state and will evolve the associated water of hydration.

Conversely, if one begins with the anhydrous lithium iodide and exposes the solid to water vapor, as long as the vapor pressure is less than any of the dissociation pressures, no hydrate phase can form. At the lowest dissociation pressure a univariant system is obtained, since upon formation of the hydrate phase there must be three phases in equilibrium. Since the experiment is being conducted at constant laboratory temperature, the pressure must also be constant. Continued addition of water vapor can only result in an increase in the amount of hydrate phase and a decrease in the amount of anhydrate phase present. When the anhydrate is completely converted, the system again becomes bivariant, and the pressure increases again with the amount of water added. The higher hydrate forms are in turn produced at their characteristic conversion pressures in an equivalent manner.

3. *Desolvated Solvates*

A desolvated solvate is the species formed upon removal of the solvent from a solvate. Depending on the empirical details of the system, the desolvated solvate may be produced as either a crystalline or an amorphous phase. These materials are not equivalent, possessing different free energies, and the amorphous phase will ordinarily be the less stable

isolating and identifying polymorphs that provides certain advantages is the availability of subsidiary patents for desirable polymorphic forms, or for retaining a competitive edge through unpublished knowledge. In 1990 Byrn and Pfeiffer found more than 350 patents on crystal forms granted on the basis of an advantage in terms of stability, formulation, solubility, bioavailability, ease of purification, preparation or synthesis, hygroscopicity, recovery, or prevention of precipitation [1].

One question that is likely to arise during the registration process is "What assurance can be provided that no other crystalline forms of this compound exist?" It is incumbent on the manufacturer of a new drug substance to show that due diligence has been employed to isolate and characterize the various solid-state forms of a new chemical entity. This may seem to be a daunting task, particularly in light of the widely quoted statement by Walter C. McCrone [2] that "Those who study polymorphism are rapidly reaching the conclusion that all compounds, organic and inorganic, can crystallize in different crystal forms or polymorphs. In fact, the more diligently any system is studied the larger the number of polymorphs discovered." On the other hand, one can take comfort from the fact that some important pharmaceuticals have been in use for many years and have, at least until now, exhibited only one stable form. Indeed, it seems to this author that there must be particular bonding arrangements of some molecules that are so favorable energetically as to make alternate arrangements unstable or nonisolatable.

In the future, computer programs using force-field optimization should be perfected to the point where it will be possible to predict, with confidence, that a particular crystalline packing arrangement is the most stable that is likely to be found. These programs also may make it possible to predict how many alternate arrangements having somewhat higher energy can potentially be isolated [3,4]. Until that time, the developmental scientist is handicapped in attempting to predict how many solid forms of a drug are likely to be found. The situation is further complicated by the phenomenon of "disappearing polymorphs" [5], or metastable crystal forms that seem to disappear in favor of more stable ones.

Some polymorphs can be detected, but not isolated. Hot stage microscopy has been used extensively to study polymorphic transfor-

are described in various USP monographs. Hydrates can be prepared by recrystallization from water or from mixed aqueous solvents. They can also result, in some instances, from exposure of crystal solvates (such as methanolates or ethanolates) to an atmosphere containing water vapor.

Crystalline substances often form with water molecules located at specific sites in the crystal lattice, which are held in coordination complexes around lattice cations. This type of water is denoted as water of crystallization and is common for inorganic compounds. For example, nickel sulfate forms a well-defined hexahydrate, where the waters of hydration are bound directly to the Ni(II) ion. Extraneous inclusion of water molecules can occur if a coprecipitated cation carries solvation molecules with it. Water also can be incorporated into random pockets as a result of physical entrapment of the mother liquor. Well-defined multiple hydrate species can also form with organic molecules. For example, raffinose forms a pentahydrate.

Although most hydrates exhibit a whole-number-ratio stoichiometry, an unusual case is the metastable hydrate of caffeine, which contains only 0.8 moles of water per mole of caffeine. Only in a saturated water vapor atmosphere will additional amounts of water be adsorbed at the surface of the 4/5-hydrate to yield a 5/6 hydrate [59].

In some instances, a compound of a given hydration state may crystallize in more than one form, so that the hydrates themselves exhibit polymorphism. One such example is nitrofurantoin, which forms two monohydrates that have distinctly different temperatures and enthalpies of dehydration. The monohydrates have quite different packing arrangements, with Form I possessing a layer structure and Form II exhibiting a herringbone motif. The included water molecules play a major role in stabilizing the crystal structures. Whereas water molecules are contained in isolated cavities in Form II, in Form I they are located in continuous channels, and this apparently facilitates the escape of water when these crystals are heated [60].

Another example of hydrate polymorphism is amiloride hydrochloride [61], which can be obtained in two polymorphic dihydrate forms. These forms are indistinguishable by techniques other than x-ray powder diffraction.

It is interesting that scopolamine hydrobromide has been reported

mations. The microscopist can detect numerous polymorphic transformations, but the individual polymorphs often prove to be so unstable that they cannot be isolated by the usual methods. An excellent example of this is the work of Grießer and Burger on etofylline [6]. These authors identified five polymorphic forms by thermomicroscopy, but only stable Modification I could be obtained by recrystallization, even when seed crystals from the hot stage were used. Similarly, Kuhnert-Brandstätter, Burger, and Völlenklee [7] described six polymorphic forms of piracetam, only three of which could be obtained by solvent crystallization. All the others were found only by crystallization from the melt. What, then, is a careful investigator to do?

In this chapter, the various methods used to isolate polymorphs, hydrates, and solvates will be described. As Bernstein [8] has observed, "The conditions under which different polymorphs are obtained exclusively or together also can provide very useful information about the relative stability of different phases and the methods and techniques that might be necessary to obtain similar structures of different chemical systems." In this context, it is hoped that the following information will prove useful in devising a "screening" protocol for the preparation of the various solid state forms of pharmaceuticals. While one cannot be absolutely certain that no additional forms will be identified in the future, this approach should provide some assurance that "due diligence" has been exercised to isolate and identify crystalline forms that are likely to arise during the normal course of drug development and storage.

A. Sublimation

On heating, approximately two-thirds of all organic compounds are converted partially from the solid to the gaseous state and back to solid, i.e., they sublime [9]. While strictly speaking the term sublimation refers only to the phase change from solid to vapor without the intervention of the liquid phase, it is often found that crystals are formed on cooler surfaces in close proximity to the melt of organic compounds when no crystals were formed at temperatures below the melting point. The most comprehensive information concerning sublimation temperatures of compounds of pharmaceutical interest can be found in tables

I. INTRODUCTION

Certainly the most important aspect relating to an understanding of polymorphic solid and solvate species is the range of analytical methodology used to perform the characterization studies [1–3]. The importance of this area has been recognized from both scientific and regulatory concerns, so the physical methods have begun to come under the same degree of scrutiny as have the traditional chemical methods of analysis. Byrn et al. have provided a series of useful definitions that concisely give the characteristics of the various solid forms that can be found for a given drug substance [4] and that will be used throughout this chapter. Compounds may be *polymorphs* (forms having the same chemical composition but different crystal structures), *solvates* (forms containing solvent molecules within the crystal structure), *desolvated solvates* (forms when the solvent is removed from a specific solvate while still retaining the original crystal structure), or *amorphous* (solid forms that have no long-range molecular order).

Of all the methods available for the physical characterization of solid materials, it is generally agreed that crystallography, microscopy, thermal analysis, solubility studies, vibrational spectroscopy, and nuclear magnetic resonance are the most useful for characterization of polymorphs and solvates. However, it cannot be overemphasized that the defining criterion for the existence of polymorphic types must always be a nonequivalence of crystal structures. For compounds of pharmaceutical interest, this ordinarily implies that a nonequivalent x-ray

powder diffraction pattern is observed for each suspected polymorphic variation. All other methodologies must be considered as sources of supporting and ancillary information; they cannot be taken as definitive proof for the existence of polymorphism by themselves alone.

In the present work, the practice of the most commonly encountered techniques performed for the solid-state characterization of polymorphic or solvate properties will be reviewed. No attempt will be made to summarize every recorded use of these methodologies for such work, but selected examples will be used to illustrate the scope of information that can be extracted from the implementation of each technique.

II. CRYSTALLOGRAPHY: X-RAY DIFFRACTION

The x-ray crystallography technique, whether performed using single crystals or powdered solids, is concerned mainly with structural analysis and is therefore eminently suited for the characterization of polymorphs and solvates. An external examination of crystals reveals that they often contain facets, and that well-formed crystals are completely bounded by flat surfaces. Planarity of this type is not commonly encountered in nature, and it was quickly deduced that the morphological characteristics of a crystal are inherent in its interior structure. In fact, the microscopic form of a crystal depends critically on structural arrangements at the atomic or molecular level; the underlying factor controlling crystal formation is the way in which atoms and molecules can pack together.

A. Single Crystal X-Ray Diffraction

Every crystal consists of exceedingly small fundamental structural units that are repeated indefinitely in all directions. In 1830, Hessel conducted a purely mathematical investigation of the possible types of symmetry for a solid figure bounded by planar faces and deduced that only 32 symmetry groups were possible for such objects. The same conclusion was reached by Bravais in 1949 and Gadolin in 1867. These 32 crystallographic point groups are grouped into six crystal systems,

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Not all instances of conformational polymorphism are as dramatic as that just described, and often different conformers of a single side chain are able to pack into different crystalline arrangements. For instance, the two polymorphs of *p*-(1*R*,3*S*)-3-thioanisoyl-1,2,2-trimethylcyclopentane carboxylic acid were found to be associated with different conformations of the carboxylate group [15]. Torsion about a single C–N bond was shown to be the origin of the polymorphism detected for lomeridine dihydrochloride [16]. Finally, relatively small differences in molecular conformation were detected for the two polymorphic and four solvated crystalline forms of spironlactone [17].

B. X-Ray Powder Diffraction

Although the solving of a crystal structure provides the greatest understanding of polymorphic solids, the necessity for obtaining suitable single crystals and the degree of complexity associated with the data analysis preclude this technique from being used on a routine basis for batch characterization. In fact, most drug substances are obtained as microcrystalline powders, from which it is often fiendishly difficult to obtain crystallographically adequate crystals. Furthermore, during the most common evaluation of drug substances, it is usually sufficient to establish only the polymorphic identity of the solid and to verify that the isolated compound is indeed of the desired structure. For these reasons, and to its inherent simplicity of performance, the technique of x-ray powder diffraction (XRPD) is the predominant tool for the study of polycrystalline materials [18] and is eminently suited for the routine characterization of polymorphs and solvates.

A correctly prepared sample of a powdered solid will present an entirely random selection of all possible crystal faces at the powder interface, and the diffraction off this surface provides information on all possible atomic spacings in the crystal lattice. To measure a powder pattern, a randomly oriented sample is prepared so as to expose all the planes of a sample and is irradiated with monochromatic x-ray radiation. The scattering angle θ is determined by slowly rotating the sample and using a scintillation counter to measure the angle of diffracted x-rays with respect to the angle of the incident beam. Alternatively, the angle between sample and source can be kept fixed, and the detector

moved along a proscribed path to determine the angles of the scattered radiation. Knowing the wavelength of the incident beam, the spacing between the planes (identified as the d-spacings) is calculated using Bragg's law.

The XRPD pattern will therefore consist of a series of peaks detected at characteristic scattering angles. These angles, and their relative intensities, can be correlated with the computed d-spacings to provide a full crystallographic characterization of the powdered sample. After indexing all the scattered lines, it is possible to derive unit cell dimensions from the powder pattern of the substance under analysis [18]. For routine work, however, this latter analysis is not normally performed, and one typically compares the powder pattern of the analyte to that of reference materials to establish the polymorphic identity. Since every compound produces its own characteristic powder pattern owing to the unique crystallography of its structure, powder x-ray diffraction is clearly the most powerful and fundamental tool for a specification of the polymorphic identity of an analyte. The USP general chapter on x-ray diffraction states that identity is established if the scattering angles of the ten strongest reflections obtained for an analyte agree to within ± 0.20 degrees with that of the reference material, and if the relative intensities of these reflections do not vary by more than 20 percent [19].

The power of XRPD as a means to establish the polymorphic identity of an analyte can be illustrated by considering the case of the anhydrate and trihydrate phases of ampicillin. The crystal structures of both phases have been obtained, and they differ in the nature of the molecular packing [20]. The amino group in the monoclinic anhydrate is hydrogen bonded to the ionized carboxyl groups of two molecules, while the amino group of the orthorhombic trihydrate is hydrogen bonded to a single carboxylate group and to the waters of hydration that link other molecules in the structure. The powder patterns of these two materials are shown in Fig. 3 and are seen to be readily distinguishable from each other. Amoxycillin trihydrate has been found to crystallize in the same space group as does ampicillin trihydrate, and it exhibits a very similar pattern of hydrogen bonding [21]. However, the dimensions of the two unit cells differ significantly, and this fact is